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Identification of a nucleic acid-binding region within the largest subunit of *Drosophila melanogaster* RNA polymerase II

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Abstract

The largest and the second-largest subunit of the multisubunit eukaryotic RNA polymerases are involved in interaction with the DNA template and the nascent RNA chain. Using Southwestern DNA-binding techniques and nitrocellulose filter binding assays of bacterially expressed fusion proteins, we have identified a region of the largest, 215-kDa, subunit of *Drosophila* RNA polymerase II that has the potential to bind nucleic acids nonspecifically. This nucleic acid-binding region is located between amino acid residues 309–384 and is highly conserved within the largest subunits of eukaryotic and bacterial RNA polymerases. A homology to a region of the DNA-binding cleft of *Escherichia coli* DNA polymerase I involved in binding of the newly synthesized DNA duplex provides indirect evidence that the nucleic acid-binding region of the largest subunit participates in interaction with double-stranded nucleic acids during transcription. The nonspecific DNA-binding behavior of the region is similar to that observed for the native enzyme in nitrocellulose filter binding assays and that of the separated largest subunit in Southwestern assays. A high content of basic amino acid residues is consistent with the electrostatic nature of nonspecific DNA binding by RNA polymerases.

Keywords: fusion proteins; nucleic acid binding; RNA polymerase II; Southwestern blotting

Transcription of DNA into RNA is accomplished by DNA-dependent RNA polymerases. RNA synthesis involves recognition and binding of transcription factors and RNA polymerase to the promoter, initiation of RNA synthesis de novo, elongation of the RNA chain processively by movement of the enzyme along the DNA template, and finally termination and release of the nascent RNA chain. The first step in transcription initiation results in the formation of binary complexes. Two kinds of binary complexes can be observed for RNA polymerases. Nonspecific binary complexes are formed at all sites of the DNA template and are nonproductive. In contrast, specific complexes are formed at promoter sites leading to the initiation of RNA synthesis (Conaway & Conaway, 1991). In eukaryotes the formation of these specific preinitiation complexes involves the interaction of several general transcription initiation factors with promoter elements and the RNA polymerases (Sawadogo & Sentenac, 1990; Sharp, 1992). The kinetics and thermodynamics of the formation of specific preinitiation complexes are influenced by a nonspecific or general binding of the enzyme to DNA; thus, the nonspecific binding is of physiological relevance (von Hippel et al., 1974). The nonspecific DNA binding of eukaryotic and bacterial RNA polymerases depends strongly on the salt concentration and is characterized by a general affinity for nucleic acids and other polyanionic substances such as heparin (Chamberlin, 1976; von Hippel et al., 1984). As shown for the *Escherichia coli* RNA polymerase, nonspecific binding of the enzyme to DNA is totally electrostatic, indicating that binding is based on the interaction of basic amino acid residues with the negatively charged phosphates of the DNA backbone (Record et al., 1976; deHaseth et al., 1978; Shaner et al., 1982; von Hippel & Berg, 1986).

Eukaryotic nuclear RNA polymerases are composed of 2 nonidentical large subunits with molecular weights between 120 and 240 kDa and about 9–13 smaller subunits (Sentenac, 1985; Young, 1991). The two large subunits of eukaryotic RNA polymerases are the structural and functional homologues of the β' and β subunit of the *E. coli*

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RNA polymerase and form the major part of the catalytic site. As shown by photochemical cross-linking experiments and antibody studies, both large subunits are involved in the interaction with the DNA template and the nascent RNA chain (Hillel & Wu, 1978; Park et al., 1980; Carroll & Stollar, 1982, 1983; Huet et al., 1982; Gundelfinger, 1983; Hanna & Meares, 1983; Bartholomew et al., 1986). Nonspecific DNA binding of the largest subunits of *Drosophila hydei* RNA polymerases I, II, and III and of RNA polymerases II of Ehrlich ascites tumor cells and of chicken myeloblastosis cells has also been demonstrated by Southwestern blotting, a technique that has been successfully used for the analysis of nonspecific as well as specific DNA binding by a large number of proteins (Bowen et al., 1980; Gundelfinger, 1983; Horikoshi et al., 1983; Chuang & Chuang, 1987; Keller & Maniatis, 1991). Nonspecific binding was also observed in Southwestern assays for the second-largest subunits of the three RNA polymerases of *D. hydei*.

The genes of a large number of RNA polymerase subunits have been cloned in the past years (for reviews see Sawadogo & Sentenac, 1990; Young, 1991). Analyzing fusion proteins expressing overlapping fragments of the largest, 215-kDa, subunit of *Drosophila* RNA polymerase II (DmRP215) by Southwestern blotting, we were able to identify a protein segment with the potential to bind DNA nonspecifically. This segment shows DNA binding behavior similar to the native enzyme in nitrocellulose filter binding assays.

Results

Nonspecific DNA binding of RNA polymerase II

Purified RNA polymerase II of *Drosophila melanogaster* was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose, and analyzed for binding of linearized plasmid DNA by Southwestern assays under standard conditions. This assay showed that both large subunits, i.e., the 140-kDa second-largest subunit and the 175-kDa IIb form of the 215-kDa largest subunit, have the potential to bind DNA (Fig. 1a). The IIb form of the largest subunit is produced by proteolytic degradation of the C-terminal heptapeptide repeat domain (CTD) found in all RNA polymerase II largest subunits (Corden, 1990). Enzymes containing this truncated largest subunit are transcriptionally fully active and can initiate RNA synthesis accurately in vitro from various promoters (Zehring et al., 1988). The second-largest subunit exhibits in these assays about 40–50% of the DNA-binding capacity of the largest subunit. Similar results have been described previously for the three nuclear RNA polymerases of *D. hydei* analyzed by Southwestern assays (Gundelfinger, 1983). No differences were observed using heat-denatured DNA and no binding was seen with free [^{32}P]dCTP. DNA binding depends strongly on the salt concentration. Increasing the NaCl concentration from 50 to 100 mM reduces the DNA binding of the largest subunit to about 58% (Fig. 1a). DNA binding is competed by preincubation of the blotted subunits with unlabeled calf liver RNA or heparin. Preincubation of 2 μg blotted RNA polymerase II with 100 μg/mL RNA results in a reduction of DNA binding to about 10%, while this value is already attained by preincubation with heparin at a concentration of 1 μg/mL. The same binding behavior, i.e., salt dependence and affinity for all kinds of nucleic acids and other polyanionic substances, can be observed for the native enzyme analyzed by nitrocellulose filter binding assays. The dependence of binding of nick-translated plasmid DNA to the enzyme on the concentration of mono- and divalent cations is shown in Figure 1b.

A nucleic acid-binding region of the largest subunit

In order to identify DNA-binding regions of the largest subunit of RNA polymerase II, overlapping β-galactosidase fusion proteins covering amino acid residues 54–1,896 were produced by cloning either genomic fragments of the DmRP215 sequence or, in the case of constructs 215-5 and 215-10 spanning introns 2 and 3, respectively, polymerase chain reaction (PCR)-amplified cDNA into pUR expression vectors (Rüther & Müller-Hill, 1983) (Fig. 2). Fusion proteins 215-1 to 215-11 were purified from inclusion bodies to about 50–80% homogeneity. All fusion proteins were recognized in immunoblot assays by two polyclonal sera generated previously against RNA polymerase II (data not shown). The fusion proteins were analyzed for nonspecific binding of nick-translated plas-

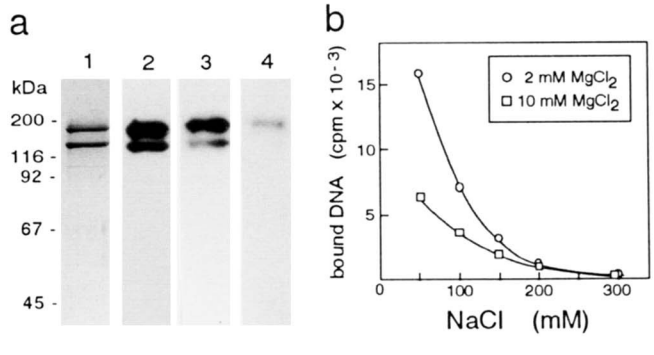

Fig. 1. a: Purified RNA polymerase II (2 μg) was separated by SDS-PAGE on a 7% polyacrylamide gel and was stained with Coomassie blue (lane 1) or was transferred to nitrocellulose and analyzed for nonspecific DNA binding by Southwestern blotting at varying salt concentrations (lane 2, 50 mM NaCl; lane 3, 100 mM NaCl; lane 4, 200 mM NaCl). The RNA polymerase II preparation used in this study yielded mostly the IIB form characterized by a proteolyzed largest subunit lacking the CTD (IIb subunit). **b:** Nonspecific binding of nick-translated linearized plasmid DNA (30,000 cpm/assay) to RNA polymerase II (100 ng) at varying NaCl and $MgCl_2$ concentrations was analyzed by nitrocellulose filter binding assays.

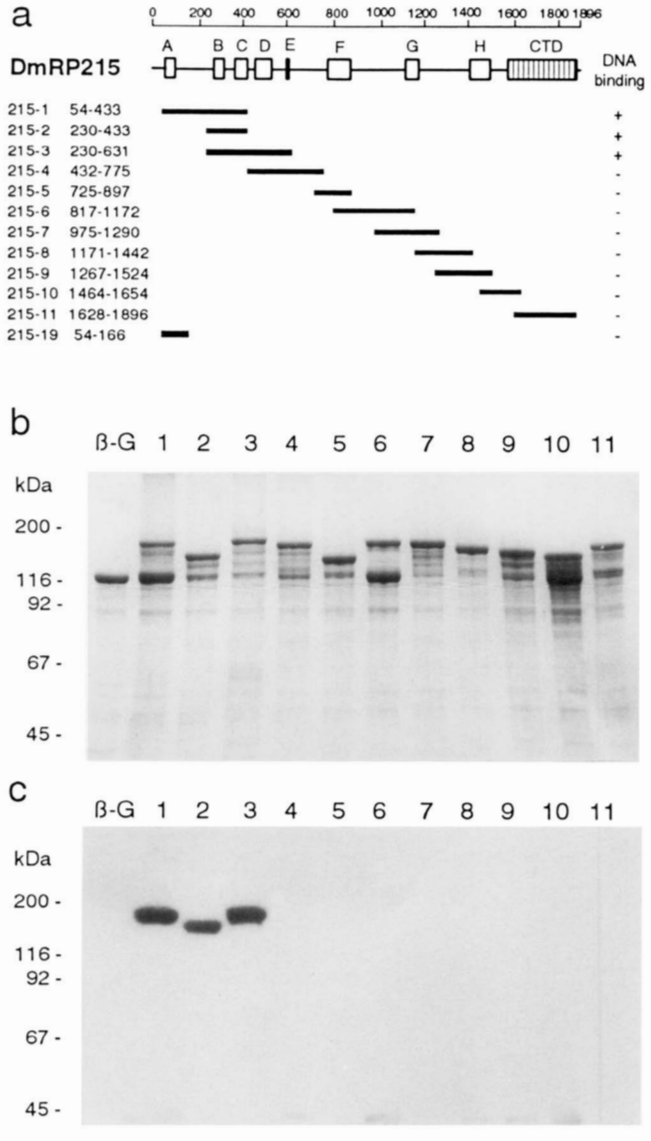

Fig. 2. Analysis of bacterially expressed β-galactosidase fusion proteins of the largest subunit (DmRP215) in Southwestern assays. **a:** Homologous regions A–H conserved within the largest subunits of RNA polymerases (Jokerst et al., 1988) as well as the C-terminal heptarepeat domain (CTD) found in the RNA polymerase II largest subunits are shown. The amino acid positions of fusion proteins 215-1 to 215-11 are indicated. Fusion proteins 215-1 to 215-11 (lanes 1–11) and β-galactosidase without a fusion part (β-G) were separated by SDS-PAGE on a 7% polyacrylamide gel and were either stained with Coomassie blue (**b**) or analyzed by Southwestern blotting for binding of nick-translated plasmid DNA under standard conditions (**c**). Three fusion proteins of the N-terminal part (215-1 to 215-3) show nonspecific DNA binding.

mid DNA in Southwestern assays under standard conditions. As shown in Figure 2 only three fusion proteins of the N-terminal region (constructs 215-1, -2, and -3; amino acids 54–631) were able to bind DNA, while β-galactosidase without a fusion part purified under identical conditions gave a negative result. No binding was observed with [^{32}P]dCTP alone. Incubation with either double-stranded or heat-denatured DNA showed no differences in the binding pattern and both substrates were bound equally well. However, the strength of DNA binding of these constructs depends strongly on the concentration of mono- and divalent cations in the binding buffer. At 100 mM NaCl the binding is reduced to about 30% and no binding was observed at 200 mM NaCl, even after prolonged exposure. An increase in $MgCl_2$ concentration from 2 to 10 mM results in a reduction of DNA binding to about 52% (Fig. 3). DNA binding was also strongly reduced by preincubation of the blotted proteins with varying concentrations of calf liver RNA, calf thymus DNA, or heparin, indicating the general affinity for nucleic acids (Fig. 3).

To localize the exact position of the nucleic acid-binding region smaller fusion proteins covering amino acid residues 309–512 (constructs 215-12 to 215-18) were produced by cloning PCR-amplified fragments (Fig. 4). Construct 215-12 expressing amino acids 309–512 exhibits a DNA-binding capacity comparable to construct 215-3 (amino acids 230–631). A segment as short as 44 amino acids (position 309–352; construct 215-17) was found to be almost sufficient for nonspecific DNA binding, showing, however, a reduced level (about 25% compared to 215-12). Extension to amino acid position 384 (construct 215-18) results in maximal DNA binding (Fig. 4). Thus, amino acids 309–384 of the largest subunit are necessary and sufficient for DNA binding. DNA binding of this re-

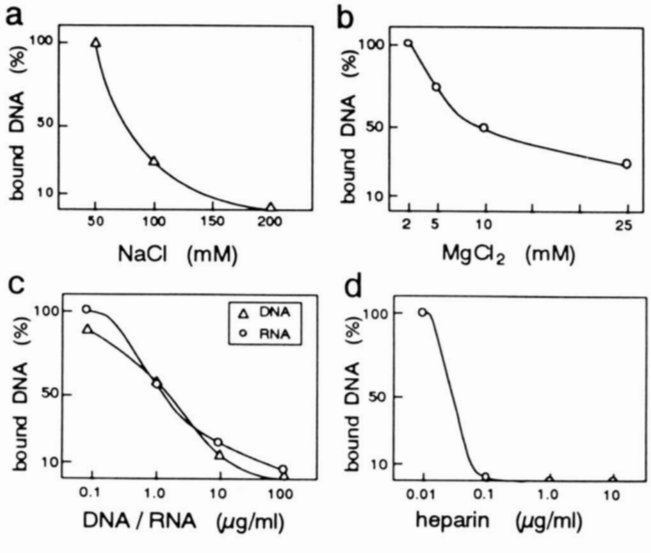

Fig. 3. Characterization of the DNA-binding behavior of fusion protein 215-3. Nonspecific DNA binding of fusion protein 215-3 (2 μg) was analyzed by Southwestern blotting at varying NaCl concentrations in the presence of 2 mM $MgCl_2$ (**a**) or at varying $MgCl_2$ concentrations in the presence of 50 mM NaCl (**b**). Bound DNA was determined by scanning densitometry of the autoradiograms. Preincubation of blotted fusion protein 215-3 with varying concentrations of calf liver RNA, calf thymus DNA (**c**), or heparin (**d**) for 1 h results in strong reduction of DNA binding.

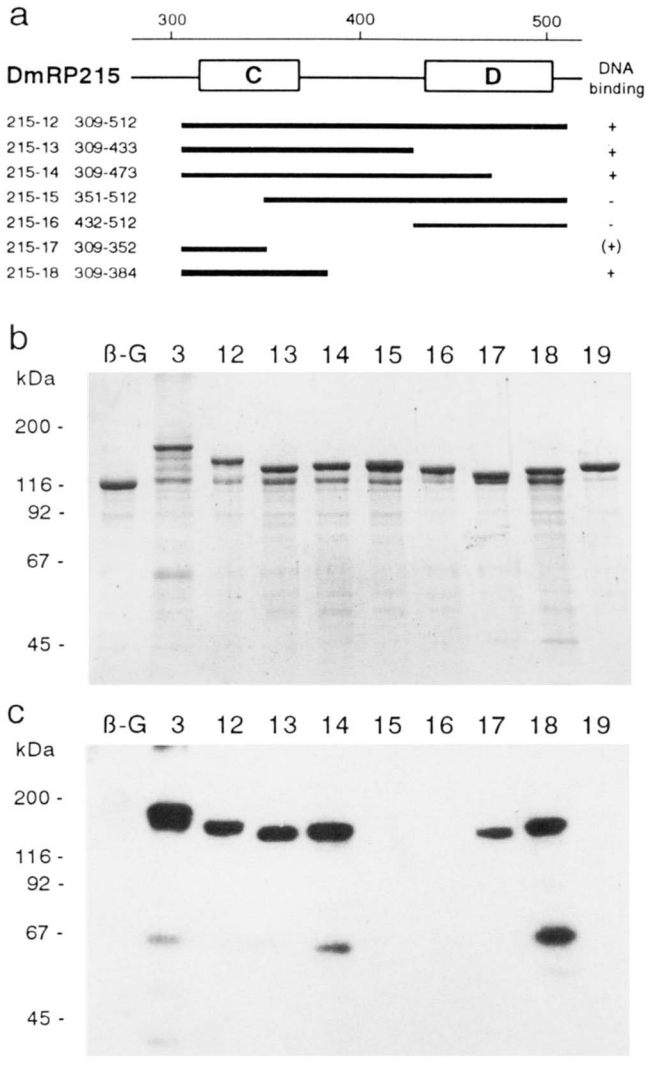

Fig. 4. Localization of a nucleic acid-binding region. **a:** The positions of fusion proteins 215-12 to 215-18 (amino acids 309–512) covering conserved regions C and D are shown. Fusion proteins 215-3 and 215-12 to 215-18 as well as fusion protein 215-19 expressing the zinc-binding motif (amino acids 54–166) were purified from inclusion bodies, separated by SDS-PAGE on a 7% polyacrylamide gel, and were either stained with Coomassie blue (**b**) or analyzed in Southwestern assays for nonspecific binding of nick-translated plasmid DNA under standard conditions (**c**). The DNA-binding protein at 60 kDa is most probably an *Escherichia coli* contaminant, since no reactivity is seen with this protein in immunoblot assays using polyclonal sera against *Drosophila* RNA polymerase II.

gion was also demonstrated by nitrocellulose filter binding assays of a maltose-binding protein (MBP) fusion protein expressing amino acids 309–512, which was purified under nondenaturing conditions, whereas MBP alone showed no DNA binding (Fig. 5).

To investigate whether construct 215-1, which contains the identified nucleic acid-binding region as well as a zinc-binding motif at amino acid position 67–83 (conserved region A; see Fig. 2), binds DNA also with this motif,

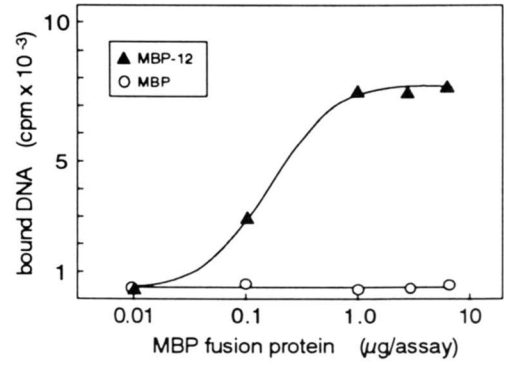

Fig. 5. Nonspecific DNA binding of an MBP fusion protein. An MPB fusion protein expressing amino acids 309–512 of the largest subunit (MBP-12) was purified under nondenaturing conditions and analyzed for binding of a 59-bp end-labeled DNA fragment (8,000 cpm/assay) in nitrocellulose filter-binding assays. MBP alone, which was purified under identical conditions, was used as negative control.

we tested an additional construct (215-19; amino acids 54–166) containing only the zinc-binding motif (Fig. 2). However, no DNA binding was observed for this fusion protein in Southwestern assays under our assay conditions in the absence or presence of DTT and zinc in the binding buffer (Fig. 4).

Structural features of the nucleic acid-binding region

The identified nucleic acid-binding region maps to region C, which is one of eight regions (A–H) highly conserved within the largest subunits of RNA polymerases (Figs. 2, 4). An alignment with the corresponding regions of the largest subunits of RNA polymerases from eukaryotes, archaebacteria, eubacteria, chloroplasts, and vaccinia virus is shown in Figure 6a. An analysis of the amino acid composition of this region shows a high content of basic amino acid residues (18%) resulting in an isoelectric point of 11.4. Regions with similar basicity were found between amino acids 90 and 140 (11.0), 440 and 480 (11.4), 960 and 1,000 (11.0), and 1,090 and 1,140 (11.4). However, these regions showed no DNA binding in Southwestern assays, indicating that a high content of basic amino acids alone is not sufficient for DNA binding.

A computer search in the SWISS-PROT database for proteins with strong sequence similarity to the nucleic acid-binding region identified only other largest subunits of RNA polymerases. Previously, Allison and coworkers (1985) have described some similarity between the largest subunits of yeast RNA polymerases II and III and the DNA polymerase I of *E. coli* and T7. Interestingly, this homology representing helix J and K of the *E. coli* DNA polymerase I lies within the identified nucleic acid-binding region (amino acids 355–380). The total nucleic acid-

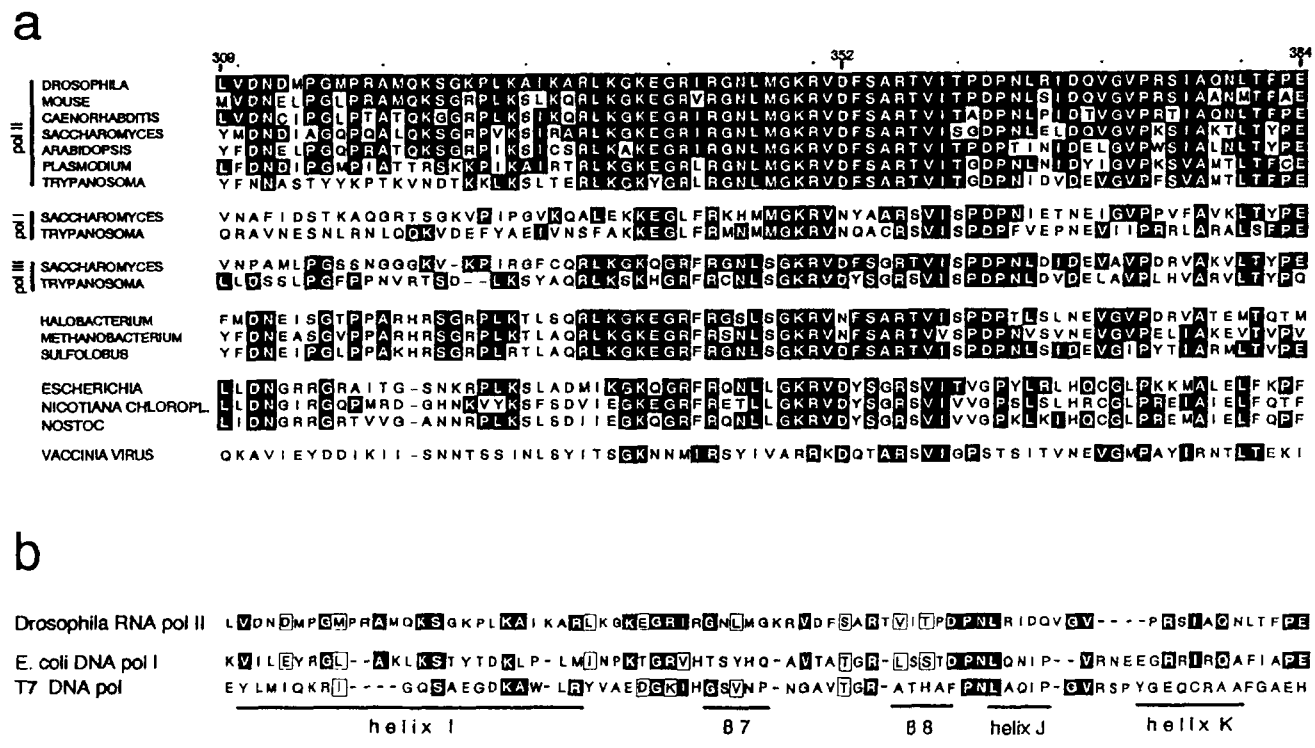

Fig. 6. a: Alignment of the nucleic acid-binding region of the largest subunit of *Drosophila* RNA polymerase II (amino acids 309–384) with the corresponding regions of the largest subunits of RNA polymerases from eukaryotes, bacteria, chloroplasts, and vaccinia virus. Identical residues are shown in black squares. Sequences were from the SWISS-PROT protein sequence database (Release 22). **b:** Similarity between the nucleic acid-binding region and DNA polymerase I of *E. coli* (amino acids 625–697) and T7 (amino acids 388–459). Identical residues are shown in black squares, and conservative exchanges (D, E) (K, R) (N, Q) (L, V, I, M) (Y, F, W) are indicated as open boxes. The positions of β-sheets and α-helices of the *E. coli* DNA polymerase I are shown (Ollis et al., 1985).

binding region shows 29% identical residues and 39% similarity, taking into account conservative exchanges (Fig. 6b). In DNA polymerase I, this region (amino acids 625–697; helix I to helix K) is part of a cleft suggested to be involved in interaction with the newly synthesized double-stranded DNA (Ollis et al., 1985).

Discussion

Using a Southwestern blotting technique we have identified a region of the largest subunit of *Drosophila* RNA polymerase II with the potential to bind DNA nonspecifically. The DNA-binding behavior of this region in Southwestern assays and nitrocellulose filter binding assays is similar to that observed for the separated subunit by Southwestern blotting and for the native enzyme analyzed by nitrocellulose filter binding assays, suggesting that DNA binding is based on the same interactions. A high content of basic amino acid residues is in agreement with an electrostatic nature of nonspecific DNA binding (deHaseth et al., 1978; Shaner et al., 1982, von Hippel et al., 1984). For the *E. coli* core enzyme it was shown that about 24 charge–charge interactions are involved in the binding of double-stranded DNA (deHaseth et al., 1978). The participation of a substantial number of lysine residues in DNA binding was demonstrated by protection of eukaryotic and bacterial RNA polymerases from reaction with various chemical reagents by preincubation of the enzyme with DNA (Krakow et al., 1976; Makoff & Malcom, 1980; Kumar, 1981). Inactivation of yeast RNA polymerase I by pyridoxal 5′-phosphate is partially protected by preincubation with DNA (Martial et al., 1975), and pyridoxal 5′-phosphate-reactive sites have been located predominantly in the largest subunit and, to a lesser extent, in the second-largest and two of the smaller subunits of yeast RNA polymerase I (Valenzuela et al., 1978).

A DNA-binding domain of the largest subunit of calf thymus RNA polymerase II was previously identified by use of the monoclonal antibody G11 (Carroll & Stollar, 1982, 1983). This antibody, which inhibits nonspecific DNA binding of the enzyme, cross-reacts with enzymes from other species and, therefore, appears to recognize a conserved epitope. This is in agreement with our finding that a nucleic acid-binding region is located in one of the evolutionarily conserved regions of the largest subunit.

The nucleic acid-binding region shows similarity to DNA polymerase I of *E. coli*. The three-dimensional

structure of the Klenow fragment has been resolved, and it was shown that this region is part of a cleft, which has been proposed by model building to bind the newly synthesized DNA double strand, projecting a two-helix motif (helices J and K) partly into the major groove (Ollis et al., 1985; Joyce & Steitz, 1987). Further evidence that amino acid residues located in or near the cleft are involved in DNA binding came from the analyses of mutations, site-directed mutagenesis, chemical modification experiments, and oligopeptide binding to DNA (reviewed in Beese & Steitz, 1989; Blanco et al., 1991). All of the positively charged residues of DNA polymerase I lie within this cleft (Warwicker et al., 1985). A weak similarity to helix J and K of the DNA polymerase I was also described for the large subunit of transcription factor TFIIE (Ohkuma et al., 1991). In their studies on the three-dimensional structure of RNA polymerases at low resolution, Darst and coworkers (1989, 1991) found a similar structure in *E. coli* RNA polymerase and yeast RNA polymerase II forming a channel of 25 Å diameter, which has the appropriate size to bind double-stranded nucleic acids. Computer-generated models showed that the DNA-binding cleft of DNA polymerase I fits well in size and shape to the proposed DNA-binding channel of *E. coli* RNA polymerase (Darst et al., 1989). The similarity of the nucleic acid-binding region to a region of DNA polymerase I involved in binding of DNA during replication led to the suggestion that the region of the largest subunit binds DNA not only in nonspecific complexes but participates also in interaction with nucleic acids in transcribing complexes.

Studies on the crystal structure of human immunodeficiency virus (HIV)-1 reverse transcriptase at 3.5 Å resolution have identified a five-subdomain arrangement of the catalytic p66 subunit forming a right-hand structure similar to that of the Klenow fragment of *E. coli* DNA polymerase I (Kohlstaedt et al., 1992), although no primary sequence similarities between these two enzymes (Kohlstaedt et al., 1992) and between reverse transcriptase and the largest subunit of RNA polymerase II were found. Model building with reverse transcriptase and the crystal structure of the Klenow fragment with duplex DNA suggest a similar role for the "thumb" subdomain in the interaction with the newly synthesized RNA-DNA hybrid or DNA double strand, respectively (Kohlstaedt et al., 1992). The region of the Klenow fragment that is homologous to the nucleic acid-binding region of largest subunit of RNA polymerase II forms part of this "thumb" subdomain. Interaction of the RNA polymerase II largest subunit with both DNA and RNA during transcription has been demonstrated by photochemical cross-linking experiments (Gundelfinger, 1983; Bartholomew et al., 1986). Further investigations will be needed to show whether the identified nucleic acid-binding region interacts with the DNA template and/or the DNA-RNA hybrid during chain elongation.

Materials and methods

Enzymes

Drosophila melanogaster RNA polymerase II was isolated in our laboratory from embryos as described (Seifarth et al., 1991). Taq DNA polymerase, RNase-free DNase I, and the Klenow fragment of *E. coli* DNA polymerase I were purchased from Pharmacia, and AMV reverse transcriptase was purchased from Stratagene. Oligonucleotide primers used for PCR were synthesized by Dr. R.W. Frank (ZMBH, Heidelberg, Germany).

Construction and purification of β-galactosidase fusion proteins

Genomic DNA fragments of the coding region of DmRP215 or PCR-amplified cDNA were cloned into pUR expression vectors (Rüther & Müller-Hill, 1983) using standard cloning techniques (Sambrook et al., 1989). Constructs 215-12 and 215-18 were produced by PCR amplification of 1 μg genomic DNA under standard conditions (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 2.5 U Taq polymerase) in a Bio-Med Thermocycler 60 using 40 pmol each of primers P215-5 (GCC GGATCCCTAGTGGACAACGATATGCC; corresponding to amino acids [aa] 309–315) and P215-6 (GCGCAA GCTTGTGATGTGGATGTTCTCCAC; aa 506–512) for construct 215-12 and primers P215-5 and P215-7 (GCGC AAGCTTACTCGGGAAGGTTAGATT; aa 378–384) for construct 215-18, respectively. *Escherichia coli* RR1ΔM15 cells containing the plasmids were grown in LB medium including 50 μg/ml ampicillin, and expression was induced with 1 mM isopropylthiogalactosidase (IPTG) at 37 °C overnight. Fusion proteins were purified from inclusion bodies by the method of Nagai and Thøgersen (1987) and were solubilized in 20 mM Tris-HCl, pH 8.0, 8 M urea, and 10 mM dithiothreitol.

Construction and purification of MBP fusion proteins

A 610-bp *Bam*HI/*Hin*dIII DNA fragment corresponding to amino acids 309–512 of the largest subunit was cloned into the pMAL™-cRI expression vector (Biolabs). The resulting MBP fusion protein as well as MBP alone were purified by maltose affinity chromatography as described by the manufacturer's protocol.

cDNA synthesis and PCR of constructs 215-5 and 215-10

RNA was isolated from adult flies by the method of Barnett et al. (1981), and poly(A)$^+$-RNA was prepared according to Lemeur et al. (1981) using poly(U)-Sepharose (Pharmacia). Poly(A)$^+$-RNA (10 μg) was digested with

DNase I and annealed with antisense primer P215-2 (GCGCAAGCTTGCATGTTCTGGAACTCAACC; aa 891–897) or P215-4 (GCGCAAGCTTTCGACTGGAAT TGCACCGTG; aa 1,648–1,654), respectively, for 6 h at 55 °C in 50 mM PIPES, pH 6.4, 400 mM NaCl, and 1 mM EDTA. After reverse transcription for 1 h at 42 °C using 10 U AMV reverse transcriptase the produced cDNA was amplified by PCR as described above using primers P215-1 (GCGCGGATCCATACTCTGCGTCAG ACGTTC; aa 725–731) and P215-2 for construct 215-5 as well as P215-3 (GCGCGGATCCACCTTCTGCTCGAT GCAGAG; aa 1,466–1,472) and P215-4 for construct 215-10.

Gel electrophoresis and Southwestern DNA-binding assays

Proteins were analyzed by SDS-PAGE according to Laemmli (1970) and were either stained with Coomassie brilliant blue R-250 (Sigma) or were electrotransferred onto nitrocellulose (Schleicher & Schuell) by semidry blotting (Pharmacia LKB) for 1 h in transfer buffer (48 mM Tris, 39 mM glycine, 0.004% SDS, 20% methanol). After blotting, filters were rinsed for 2 × 15 min in 6 M urea with 0.2% Nonidet P-40 and washed 3 × 20 min in standard DNA-binding buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 2 mM $MgCl_2$, 50 μM $ZnCl_2$, and 0.1% NP-40). Filters were incubated in binding buffer for 1 h at room temperature with linearized pBluescript-DNA (10^5 cpm/mL) labeled with [α^{32}P]dCTP (3,000 Ci/mmol; Amersham-Buchler) by nick translation (Gibco-BRL), and were finally washed 3 × 10 min in binding buffer (Bowen et al., 1980; Gundelfinger, 1983). After drying, filters were exposed overnight to Kodak X-Omat AR5. Binding efficiency was analyzed by scanning densitometry. The efficiency of protein transfer was determined by scanning densitometry of amido-black-stained filters (Treich et al., 1991). For competition experiments blotted proteins were preincubated with varying concentrations of unlabeled calf liver RNA, calf thymus DNA (both from Sigma), or heparin (Serva) in standard binding buffer for 1 h followed by three washes in binding buffer.

Nitrocellulose filter-binding assays

DNA binding of purified RNA polymerase II (0.1 μg/assay) or of MBP fusion proteins was analyzed by nitrocellulose filter-binding assays as described by Carroll and Stollar (1982) using the following binding buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 2 mM $MgCl_2$, and 100 μg/mL bovine serum albumin) and nick-translated, linearized pBluescript DNA (30,000 cpm; specific activity ~10^7 cpm/μg) or a 59-bp DNA fragment, which was end-labeled according to Sambrook et al. (1989) using the Klenow fragment of *E. coli* DNA polymerase I.

Computer analysis of protein sequences

Sequence analyses were performed with the HUSAR program (DKFZ, Heidelberg, Germany) and the PC/GENE program (IntelliGenetics) using the SWISS-PROT protein sequence database (Releases 17 and 22).

Acknowledgments

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